Original research article

***Cichorium intybus* attenuates Streptozotocin-induced pancreatic β -cell damage by inhibiting NF- κ B activation and oxidative stress**

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Abstract

The aqueous extract of *Cichorium intybus* (CIE) leaves have shown the properties of protecting against pancreatic β -cell damage by streptozotocin (STZ), but the molecular mechanisms of its protection are not completely elucidated yet. Our current study focuses on elucidating the mechanisms of these preventive effects of CIE in MIN6 cells and an *in-vivo* model of Wistar rats. CIE offers protection against STZ in MIN6 cells by reducing the pro-oxidants and increasing the activity of the antioxidant enzymes. *In vitro* results also indicated that CIE inhibited cytotoxicity, reduced Reactive oxygen species (ROS), maintained glucose-stimulated insulin secretion and reduced NF- κ B p65 translocation into the nucleus. The group administered with a 250 mg/kg dose of CIE *in vivo* has shown an ability to maintain blood glucose level and also to preserve the number and morphology of pancreatic islets when compared to the diabetic group treated with STZ. Probably, active compounds like quercetin, rutin, and catechin present in CIE, preserve the integrity of pancreatic islets thereby protecting β -cells from the adverse effects of STZ.

Keywords: *Cichorium intybus*; Cytotoxicity; MIN6; NF- κ B; pancreatic β -cell; Streptozotocin

Highlights:

- *Cichorium intybus* attenuates pancreatic β -cell damage *in vitro* and *in vivo*.
- *Cichorium intybus* reduces NF- κ B p65 translocation into nucleus.
- *Cichorium intybus* enhances glucose induced insulin secretion and promotes ROS reduction.
- *Cichorium intybus* reduces the cytotoxicity of streptozotocin.

Introduction

Diabetes mellitus (DM) is recognized as the fourth most commonly diagnosed chronic condition (Hsiao et al., 2014). This multifactorial disease has become a major threat to worldwide health. The prevalence of metabolic syndrome and obesity is observed in type 2 diabetes. Diabetes also occurs as a result of oxidative stress, due to increased free radical production or decreased antioxidant capacity, both of which fail to scavenge the free radicals (Nambirajan et al., 2018).

Streptozotocin (STZ) is a β cell-specific toxin that induces the formation of superoxide anions in mitochondria, which in turn inhibits the Krebs cycle, substantially decreasing O_2 consumption through mitochondria and limiting ATP production and so depleting the nucleotides in β -cells (Kakkar et al., 1998). It has been proven that STZ induces H_2O_2 generation, which leads to DNA fragmentation in pancreatic β -cells *in vitro* and *in vivo* (Takasu et al., 1991).

NF- κ B, a transcription factor that influences multiple unrelated phenotypic traits, controls the expression of several inflammatory genes which are thought to create a pre-diabetic inflammatory response (Fan et al., 2018; Lu et al., 2014). In stress-induced pancreatic β -cell, the activated NF- κ B complex translocates into the nucleus and influences target genes such as NOS2A (nitric oxide synthase inducible), MAP4K1 (MAP kinase), SOD2 (superoxide dismutase 2, mitochondrial), etc. (Kanarek et al., 2010). However, it is unclear whether oxidative stress induced by STZ is mediated through NF- κ B activation or through ameliorating oxidative stress in the pancreatic β -cell. Since, sustained activation of NF- κ B by hyperglycemia has been observed in several experimental systems (Chen, 2005) and also inducing oxidative stress (Rezagholidzadeh et al., 2016).

The traditional healers are using *Cichorium intybus* (*C. intybus*), a chicory plant of the family Asteraceae for the treatment of type 1 and type 2 diabetes. The mechanism by which this plant extract heals diabetes is not clear yet. *C. intybus* has

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been reported to have an anti-hepatotoxic property by preventing the peroxidation of lipids, thereby lowering glucose-6 phosphatase activity (Pushparaj et al., 2007), by modulating cytokine secretion (Karimi et al., 2014), through inhibition of NLRP3 inflammasome activation (Shim et al., 2016), which are all highly relevant to biochemical parameters in diabetes. The present study focuses on elucidating the inhibitory mechanism of aqueous extract of *C. intybus* in STZ induced pancreatic β -cell damage. We aimed to demonstrate that the inhibitory mechanism could be mediated by the reduction in an *in vitro* NF- κ B translocation and also to experiment pancreatic β -cell damage reduction through *in vivo* studies.

Materials and methods

Materials

Streptozotocin was obtained from Sigma, St Louis, USA. Dulbecco's Modified Eagles medium (DMEM) was purchased from Thermo Scientific, Waltham, USA while Fetal bovine serum (FBS) and trypsin-EDTA solution were obtained from GIBCO, USA. Dichlorofluorescein diacetate (DCF-DA), 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), protease inhibitor were obtained from Sigma, USA. BrdU proliferation kit (QIA58) was obtained from Calbiochem, USA. Labserv insulin ELISA kit (DKT076) was obtained from Thermo Scientific, USA. Anti- β actin, anti- CDK 2, anti- goat IgG conjugated with horse-radish peroxidase and nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma, USA. Anti- NF- κ B p65 was obtained from Santa Cruz, USA. All other chemicals were of analytical grade.

Preparation plant extracts

Leaves of *C. intybus* L., (a chicory plant locally called Kasini) were collected from Vaniyampadi Village, Vellore District, Tamil Nadu, India and were authenticated and deposited at the Plant Anatomy Research Centre, Tamil Nadu with Voucher number PARC/2016/3271. These leaves were shade dried, ground and 250 g of grounded leaf powder was mixed with 300 ml of solvent and extracted through a rotary evaporator. Sequential extraction method, from low polar to high polar solvents such as Hexane (Hex), Dichloromethane (DCM), Ethyl acetate (EA), Methanol (Met) and Water (Aq/aqueous) was followed using Soxhlet apparatus (Muthusamy et al., 2008) and the extracts were freeze-dried.

Culturing of MIN6 cells and experimental design

MIN6 cells (Mouse insulinoma cells) were purchased from the National Centre for Cell Sciences (NCCS), Pune, India and grown at 37 °C in DMEM medium containing 10% FBS with 2 mM L-glutamine, 100 IU/ml of penicillin, 100 U/ml penicillin/streptomycin, 2.5 μ g/ml of amphotericin B under a humidified 5% CO₂ atmosphere and maintained at pH 7.4. The phenotype of MIN6 cells was tested to check their ability to secrete insulin in low (9.9 mM) and high glucose (35 mM) medium (data not shown). Streptozotocin (STZ) in a final concentration of 5 mM was used to induce β -cell damage. Our preliminary studies revealed that, among the five different solvent extracts of *C. intybus*, the aqueous extract resulted in high antioxidant activity and so the treatment with aqueous extract was carried out with two different concentrations (5 μ g/ml to 100 μ g/ml) and two time periods (24 h and 48 h). Based on these results, a period of 24 h and two different concentrations (5 μ g/ml and 50 μ g/ml) of the aqueous extract were chosen for the further experiments. The extract was added to the cells

along with 5 mM STZ. A control was treated with 50 μ g/ml aqueous extract of *C. intybus* alone. After 24 h treatments, the cells were subjected to sonication (Lark Bandelin electronic, UW 2070 type, Germany) for the assessment of pro-oxidant and antioxidant enzymes. For western blotting, the cells were trypsinized, and the cytosolic and nuclear fractions were separated to study the translocation of NF- κ B. All the experiments were done in triplicates.

High-Performance Liquid Chromatography – photodiode array (HPLC-PDA)

Chromatogram of the aqueous extract of *C. intybus* was performed using Agilent 1260 series HPLC with PDA detector G1315D, autosampler G1329B with pump G1311C using C18 column (ZORBAX Eclipse plus C18 analytical column 4.6 $\times$ 250 mm with 5-micron particle size) with the injection volume of 20 μ l in the mobile phase of CH₃CN/H₂O from 0–100% of CH₃CN for 50 min and detection at 260 nm in a flow rate of 0.5 ml/min (Naczek and Shahidi, 2004). Plant polyphenols like quercetin, catechin and rutin were run as standards. All samples were syringe filtered before analysis and were run in triplicates.

MTT assay for cell proliferation

1 $\times$ 10⁴ MIN6 cells were seeded in 96 well culture plate and were incubated in the CO₂ incubator till they were 80% confluent. After 24 h of treatment, the reduction of MTT to formazan was determined for cell viability (Madesh and Balasubramanian, 1997).

5-Bromo-2-deoxyuridine (BrdU) – labeling cell proliferation assay

BrdU cell proliferation assay kit (Calbiochem, CA, USA) was used to measure the incorporation of BrdU during DNA synthesis according to the manufacturer's protocol, and the absorbance was measured at 405 nm using a microtitre plate reader (BIO-RAD, Model 680, USA).

Assessment of cell death – by Trypan blue dye exclusion

1 $\times$ 10⁴ MIN6 cells were seeded in 96 well culture plate and were incubated in the CO₂ incubator to attain confluence. After the treatment, the viability was assessed (Strober, 2001).

Assessment of cell cytotoxicity – lactate dehydrogenase release assay

The activity of the LDH enzyme, both in the medium and in the cells, was estimated by following the protocol of Moss et al. (1986).

Assessment parameters of oxidative stress

Levels of thiobarbituric acid (TBA) reactive products were determined as per Ohkawa et al. (1979), protein carbonyl content according to Sohal et al. (1993), nitric oxide as per Ignarro et al. (1987), xanthine oxidase (XOD) and xanthine dehydrogenase (XDH) according to protocol of Parks and Granger (1988) were assessed. Total protein content was estimated by Bradford's method (Bradford, 1976).

Assessment of antioxidant enzymes

The activities of catalase (CAT) (Aebi, 1984), glutathione-S-transferases (GSTs) (Awasthi et al., 1980), glutathione reductase (Racker, 1955) and superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971) were analyzed.

Glucose-stimulated insulin secretion

MIN6 cells were cultured in 6 well plate, and after the treatment, the cells were washed three times with Krebs-Ringer bicarbonate buffer and incubated for 1 h at 37 °C with 9.9 mM glucose or 35 mM glucose. Aliquots were used for insulin assay (Latha et al., 2004). Insulin was quantified using the Labserv insulin ELISA kit (Thermo Scientific, USA).

ROS detection by DCFH -DA

Fluorescence level of DCF is directly proportional to the ROS in the cell and the sample was excited at 485 nm, and the absorbance was measured at 530 nm using FACS (BD FACS Calibur™) (Eruslanov and Kusmartsev, 2010).

Western blotting for the expression of NF-κB p65 in nuclear and cytosolic extracts of MIN6 cells

Cytosolic and nuclear extracts were prepared as per the method described by Deryckere and Gannon (1994). Briefly, 50 µg proteins of the cytosolic and nuclear extracts were separated by 12% SDS-PAGE, and the amount of NF-κB p65 was measured. β-actin and CDK2 were used as a loading control for cytosolic and nuclear extract respectively. Separated proteins were blotted onto PVDF membrane and were blocked with 2% BSA and were incubated with 1 µg/ml of primary antibody for overnight at 4 °C. After washing, it was allowed to react with horseradish peroxidase-conjugated IgG for 2 h at 37 °C. TMB quantified the level of protein expression, and the image was captured using a ChemiDoc™ XRS+ molecular imager (BIO-RAD, molecular imager, USA).

In vivo study: Experimental animals

Male Wistar rats around 130–170 g were selected for the study. Animals were housed under standard laboratory conditions as per Guide laboratory animals for the care and use (National Research Council, 2011). Rats were obtained from the Central Animal House, Kovai Medical Centre and Hospital (KMCH) College of Pharmacy, Coimbatore, Tamil Nadu, India. The present study was performed following institutional ethics guidelines for the care of laboratory animals (Ethical clearance number: KMCRET/PhD/11/2013-14).

Experimental design

About 25 rats were randomized into five groups ($n = 5$) as follows:

Group 1: Normal control (NC), Group 2: Diabetic rats (DC), Group 3: DC+ Glibenclamide (5 mg/kg), Group 4: DC+ CIE at 125 mg/kg dose and Group 5: DC+ CIE at 250 mg/kg dose.

The animals fasted for 12 h before blood sampling. The blood sample was collected using a tail vein puncture method, and glucometer strips were used to measure the blood glucose level. The blood glucose level of animals was estimated, before the injection of STZ in 0.05 M citrate buffer on days 3, 7, 14, 21, and 28. The rats were sacrificed on the 29th day after the completion of the study. Blood samples were collected from their eye orbital plexus (Pushparaj et al., 2007).

Experimental diabetes induction and design

Diabetes was induced by streptozotocin 60 mg/kg through intraperitoneal injection in cold citrate buffer (0.1 M, pH 4.5) in the overnight fasted rats (Pushparaj et al., 2007). The induction of diabetes was verified after 72 h by using a glucose meter (Glucocard 01-mini, Arkray Factory Inc., Japan). Diabetic rats showing blood glucose greater than 250 mg/dl were selected to assess the activity of CIE. Glibenclamide and CIE were administered orally to the diabetic groups 3, 4 and 5 respectively.

Assessment of histopathology of the pancreas in rats

The rats were sacrificed, and their pancreas was excised and fixed in 10% formalin solution to perform histopathology of the pancreas (Antu et al., 2014). The sections of the pancreatic tissues were stained with hematoxylin and eosin and examined at a 400× light microscope. The criteria for scoring the islet cell destruction were as follows: score 0 was considered for normal cells without any damage, and scores 1, 2, 3 and 4 were considered for mild, moderate, serious and severe damages, respectively.

Statistical analysis

Data obtained were analyzed using ANOVA, with Bonferroni correction for multiple t -tests as a posthoc test, to look for differences in the mean. The $p < 0.05$ was taken to indicate significance. Data analysis was carried out using SPSS, version 11.

Results

HPLC chromatogram of the aqueous extract of Cichorium intybus and identification of compounds

Reverse-phase HPLC chromatogram fingerprints of aqueous extracts of *C. intybus* were obtained at 260 nm. The aqueous extract showed the presence of 6 peaks at various retention times as shown in Fig. 1. Among these, the retention times at 10.9 minutes, 18.3 minutes and 20.8 minutes correspond to polyphenolic standards of rutin, quercetin and catechin, respectively. As per the HPLC chromatogram of aqueous extract of *C. intybus* leaves (1 mg/ml), rutin constitutes 0.6 mg/ml, quercetin constitutes 0.25 mg/ml and catechin constitutes 0.09 mg/ml with all other unknown constituents that are not observed with the significant peaks and could be in negligible amount.

Assessment of cell proliferation

5 mM STZ treatment of MIN6 cells resulted in a significant decrease in proliferation ($27 \pm 0.05\%$) when compared to untreated control cells (Fig. 2a). Cells treated with CIE (5 µg/ml and 50 µg/ml) along with 5 mM STZ have shown statistically significant increase in viability ($76 \pm 0.1\%$ and $86 \pm 0.1\%$ respectively) when compared to STZ treatment alone.

These results were further confirmed by BrdU incorporation. 5 mM STZ treatment significantly reduced cell proliferation (absorbance 0.09 ± 0.02 vs. absorbance of control cells 0.5 ± 0.1) – Fig. 2b. Cells treated with CIE (50 µg/ml) along with 5 mM STZ have considerably increased proliferation compared to 5 mM STZ.

Assessment of cell death

STZ reduced the viability of cells to $66 \pm 6\%$ when compared to the untreated control group ($93 \pm 4\%$) shown in Fig. 2c. CIE treated groups have shown a significant increase in viability to $88 \pm 4\%$ and $91 \pm 3\%$ for 5 µg/ml and 50 µg/ml concentration, respectively.

Assessment of cell cytotoxicity

In the LDH assay, the treatment with 5 mM STZ leads to a significant increase in cytotoxicity in MIN6 cells. It is indicated by a reduced LDH activity in cell lysate and a concurrently increased LDH activity in the conditioned medium of the STZ treated cells (compared to the untreated control group) – Fig. 2d. STZ + CIE (50 µg/ml) treated cells differed in their LDH activity significantly from the cells treated with STZ alone. This

result implies that the cell death induced by STZ could be due to cytotoxicity which could be overcome by CIE.

Studies on the pro-oxidant system

A significant increase in levels of TBARS (Fig. 3a), protein carbonyl content (Fig. 3b), nitric oxide content (Fig. 3c) and xanthine oxidase (Fig. 3d), and a decrease in the activity of xanthine dehydrogenase (Fig. 3e) were observed in the cells treated with STZ. CIE normalized the levels of all the enzymes in a dose-dependent manner. A significant decrease in the pro-oxidant levels and an increase in the activity of xanthine dehydrogenase were noted in the cells treated with 50 µg/ml of CIE. Nitro-L-arginine methyl ester (L-NAME) is a nitric oxide inhibitor used as a control for nitrate assay (Fig. 3c) to compare CIE to STZ treated cells.

Fig. 1. HPLC/UV profile of (a) Aqueous extract of *Cichorium intybus* L., (b) Catechin, (c) Quercetin, (d) Rutin detected at 260 nm in a flow rate of 0.5 ml/min.

Studies on antioxidant enzymes

Activities of CAT (Fig. 4a), glutathione reductase (Fig. 4b), GST (Fig. 4c) and SOD (Fig. 4d) were decreased significantly in the cells treated with 5 mM STZ when compared to the untreated control cells. Simultaneously, a significant increase in these enzyme activities was noted in cells co-treated with 50 µg/ml of CIE. Except for GST, the increase was significant for 5 µg/ml of CIE, too.

Assessment of glucose-induced insulin secretion

A dose-dependent increase in the level of insulin secretion was observed in the cells treated both with 5 µg/ml and 50 µg/ml of CIE + 5 mM STZ in both high and low glucose stimulation as compared to cells treated with 5 mM STZ alone (Fig. 5).

Detection of reactive oxygen species

As illustrated in Fig. 6a and 6b, the treatment of MIN6 cells with 5 mM STZ resulted in a significant increase of ROS positive cells. When co-treated with CIE (50 µg/ml), the percentage of ROS positive cells was similar to control and significantly decreased compared to STZ alone treated group. Concerning 5 µg/ml of CIE treatment, no significant decrease in the percentage of ROS positive cells was observed. Fig. 6b represents the histogram of the percentage of ROS levels in each group based on a shift in fluorescent intensity towards the M1 population.

Assessment of translocation of NF-κB from the cytosol to the nucleus

Next, the level of p65 in the cytoplasm and nucleus was measured. As expected, upon 5 mM STZ treatment, the level of p65 declined in the cytoplasm to 30% of its level in control cells, and concurrently increased in the nucleus to 250% of its level in control cells (Fig. 7). However, the cells treated with 50 µg/ml of CIE + 5 mM STZ have significantly reduced the translocation of p65 (Fig. 7) compared to cells treated with 5 mM STZ alone. These results showed that CIE inhibits the STZ induced translocation of p65 to the nucleus, and this is consistent with the inhibition of STZ by CIE.

Effects of CIE on fasting blood glucose

From the 14th day onwards, the 250 mg/kg CIE treated rats displayed significant ($p < 0.005$) reduction of hyperglycemia compared to diabetic control rats (Fig. 8). In this dose, CIE showed to be as effective as glibenclamide (5 mg/kg body weight) on the 21st and 28th day while compared to the diabetic control rats. Rats treated with 125 mg/kg CIE reached a significantly reduced hyperglycemia (compared to the untreated diabetic rats) only on the 28th day.

Histopathology of the pancreas

The sections of the pancreas from the untreated diabetic rats (Group 2) revealed an extensive inflammation of islet cells when compared to that of the normal healthy control rats with a score value of 3 versus 0 (Fig. 9). Further, there was a definite reduction in the number of islets in diabetic rats, compared to that of normal control rats. The size of the islets has been extensively reduced in group 2 in comparison to group 1. The size of the islets has been maintained in groups 3 and 5. The sections from CIE treated diabetic rats obtained a score value of 2 and 1 on 125 mg/kg and 250 mg/kg of CIE treatment and with the score 1 for Glibenclamide (5 mg/kg) treated group.

Fig. 2. Effect of CIE on 5 mM STZ treated MIN6 cells. (a) MTT formazan formation, (b) BrdU cell proliferation, (c) Trypan blue dye exclusion, (d) LDH cell cytotoxicity. Assayed in control, 5 mM STZ, 5 mM STZ + 5 µg/ml CIE, 5 mM STZ + 50 µg/ml CIE and CIE 50 µg/ml alone. The columns represent the mean $\pm$ SD, $n = 6$. * symbol represents groups that differed significantly from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from 5 mM STZ control group ($p < 0.05$).

Fig. 3. Effect of CIE on a pro-oxidant system in MIN6 cells. Levels of (a) TBARS, (b) Protein carbonyl content, (c) Nitric oxide, (d) Xanthine oxidase, and (e) Xanthine dehydrogenase were studied in the untreated control, 5 mM STZ, 5 mM STZ + 5 µg/ml CIE, 5 mMSTZ + 50 µg/ml CIE and 50 µg/ml CIE alone. The columns represent the mean $\pm$ SD, $n = 3$. * symbol represents groups that differed significantly from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from 5 mM STZ treated control group ($p < 0.05$).

Fig. 4. Effect of CIE on levels of antioxidant enzymes secretion in STZ induced β pancreatic cell damage in MIN6 cells. Levels of (a) Catalase, (b) Glutathione reductase, (c) Glutathione-S-transferase, and (d) Superoxide dismutase were studied in the untreated control, 5 mM STZ, 5 mM STZ + 5 μ g/ml CIE, 5 mM STZ + 50 μ g/ml CIE and 50 μ g/ml CIE alone. The columns represent the mean $\pm$ SD, $n = 3$. * symbol represents groups that differed significantly from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from 5 mM STZ control group ($p < 0.05$).

Fig. 5. Effect of CIE on glucose-induced insulin secretion. The columns represent the mean $\pm$ SD, $n = 3$. * symbol represents groups significantly differ from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from 5 mM STZ control group ($p < 0.05$).

Fig. 6. Effect of CIE on ROS production. (a) Representation of FACS histograms depicting ROS-dependent DCFDA fluorescence in MIN6 cells without DCFDA (Unlabelled, Blackline), without treatment (control, blue line), 5 mM STZ treatment (red line), 5 mM STZ and CIE (5 and 50 µg/ml; yellow and pink line), CIE (50 µg/ml; green line). (b) The columns represent the percentage gated in the M2 region (positive for DCF) with $n = 3$ independent experiments, the percentage of ROS positive in MIN6. * symbol represents groups significantly differed from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from 5 mM STZ control group ($p < 0.05$).

Fig. 7. Effect of CIE on western blotting of proteins extracted from MIN6 cells. (a) Western blot showed a major band of 65 kDa corresponding to the expected molecular weight of NF-κB p65 in the cytosol and nucleus. β actin and CDK-2 were used as internal loading controls for cytosol and nucleus, respectively. (b) The columns represent the normalization of NF-κB p65 concerning the loading control through relative intensity. Each column represents the mean $\pm$ SD, $n = 3$. * symbol represents groups that differed significantly from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from 5 mM STZ control group ($p < 0.05$).

Fig. 8. Effect of CIE on diabetic rats. Fasting blood glucose concentration (FBG) in STZ induced diabetic rats for 28 days. Data are expressed as mean $\pm$ SEM (group). From the 14th day onwards, the fasting blood glucose concentration of CIE (250 mg/kg) treated rats was significantly different from the fasting blood glucose concentration of untreated diabetic rats (Group 2). Each column represents the mean $\pm$ SD, $n = 5$. * Symbol represents groups that differed significantly from the untreated control group ($p < 0.05$). # symbol represents groups that differed significantly from the diabetic control group treated with 60 mg/kg of STZ ($p < 0.05$).

Fig. 9. Histopathology of the pancreatic tissue stained with hematoxylin and eosin (400 $\times$). **(A)** Normal control rats, islets of Langerhans with normal islet cell population. **(B)** Diabetic control rats, an islet with few preserved islet cells and infiltration by inflammatory cells. **(C)** Glibenclamide (5 mg/kg) treated diabetic rats, maintained the pancreatic islet cells with prominent islets. **(D)** CIE treated (125 mg/kg) diabetic rats with a mildly reduced number of islet cells. **(E)** CIE treated (250 mg/kg) diabetic rats with, restoration of pancreatic islet cells with prominent islets.

Discussion

Type 1 diabetes mellitus is an autoimmune disease that results in the destruction of β -cells in the islets of Langerhans. Various studies have suggested that mitochondrial reactive oxygen species (ROS) induced by high glucose play a significant role in causing pathogenesis of diabetes mellitus and its complications through the modification of several mitochondrial events (Nishikawa and Araki, 2008). Here we showed that STZ induced ROS production is ameliorated by the anti-oxidative effect of CIE that resulted in a decrease of cytotoxicity. Furthermore, CIE restored the levels of SOD, catalase, glutathione reductase and GST activities, which is in concurrence with the similar report in STZ treated mice (Adachi et al., 2011), where cellular components such as lipids, proteins, and DNA were reported to be extensively damaged. Similarly, Glutathione S-reductase (GR) and GST depletion in pancreatic β cells can be restored by the CIE treatment. Also, it was observed that an increase in TBARS, protein carbonyl content, nitric oxide, xanthine oxidase and a decrease in xanthine dehydrogenase, glucose-induced insulin secretion by STZ can be restored upon treatment with aqueous extract of CIE. The observed results correlate with a study on the effect of KIOM-4 in SOD activity in rat pancreatic tissues by Rathore et al. (2000). Enhancement of antioxidant enzymes activity and reduction in malondialdehyde could be the primary mechanisms of CIE action in our studies, as a similar mechanism was reported earlier in CCl₄-induced liver fibrosis in mice (Li et al., 2014).

NF- κ B, an oxidative responsive transcription factor, can be activated by various stimuli including hyperglycemia, an elevated level of free fatty acids, ROS, pro-inflammatory cytokines, oxidized LDL and DNA damage (Evans et al., 2002).

In the current study, the translocation of the NF- κ B subunit p65 into the nucleus was evident during STZ treatment, but the concurrent CIE treatment resulted in the inhibition of p65 translocation. This result suggests that this could be one of the mechanisms by which CIE prevents β -cell damage caused by STZ and can also be implicated as a relevant pancreatic β -cell survival factor in the presence of STZ. Rezagholizadeh et al. (2016) reported that some aqueous chicory seed extract components, such as caffeic acid, may directly inhibit the binding of NF- κ B with its DNA binding sequence and other components of *C. intybus*, that are capable of undergoing reduction-oxidation cycling, may interfere by DNA binding of NF- κ B indirectly.

The stress induced by STZ is likely to be involved in the progression of pancreatic cell dysfunction found in diabetes. Pancreatic cells are damaged due to STZ induced oxidative stress (Antu et al., 2014). The integrity of the pancreas is responsible for the normal insulin secretion. The histopathology results of the pancreas in diabetic control (DC) revealed the destruction of pancreatic cells along with moderate inflammation. The group treated with 250 mg/kg of CIE has almost maintained the pancreatic cell mass and islet size compared to that of diabetic control. The size of the islets in the group treated with 125 mg/kg of CIE was maintained to a certain extent but not equivalent to group 3 and 5. The Fasting Blood Glucose (FBG) has been maintained and found to be decreased in the groups treated with CIE as shown earlier (Pushparaj et al., 2007). An increase in antioxidant enzymes activity and reduction of pancreatic β -cell damage by ameliorating oxidative stress probably could be mainly due to the presence of quercetin, catechin, and rutin in the aqueous extract of *C. intybus*. A comprehensive review of chemical constituents and their pharmacological activities of *Cichorium* has been published recently (Aisa et al., 2020).

Conclusions

Based on the results of the study, it can be concluded that STZ-induced oxidative stress is mediated through activation of NF- κ B. CIE treatment resulted in increased antioxidant enzyme activities and reduction in pro-oxidant levels. We postulate that this could be the primary mechanism of action of CIE in the prevention of STZ induced pancreatic β -cell damage in MIN6 cells. Using an animal model, this study further demonstrated that CIE treatment has decreased the level of blood glucose and reduced the inflammation in the pancreas when compared to 60 mg/kg STZ treatment group. In summary, we conclude that CIE could prevent oxidative stress-induced β -cell damage, which can further prevent inflammation in the pancreas and restore the integrity and functionality of the pancreas. Further studies are however warranted to extrapolate the results to prove that CIE can ameliorate oxidative stress-induced β -cell damage other than STZ.

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Conflict of interests

The authors declare no conflict of interests.

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